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Review

The method controls the story - Sampling method impacts on the detection of pore-water nitrogen concentrations in streambeds

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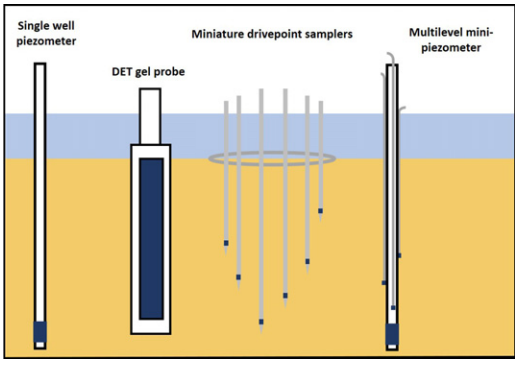
HIGHLIGHTS

- Many sampling methods exist to sample streambed biogeochemistry
- There is a lack of systematic protocols for sampling methodologies
- Sampling techniques were reviewed and investigated in a case study
- Porewater NO_3^- did not differ significantly between sampling methods but NH_4^+ did
- 'Active' versus 'passive' sampling methods may affect results

GRAPHICAL ABSTRACT



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ABSTRACT

Biogeochemical gradients in streambeds are steep and can vary over short distances often making adequate characterisation of sediment biogeochemical processes challenging. This paper provides an overview and comparison

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of streambed pore-water sampling methods, highlighting their capacity to address gaps in our understanding of streambed biogeochemical processes. This work reviews and critiques available pore-water sampling techniques to characterise streambed biogeochemical conditions, including their characteristic spatial and temporal resolutions, and associated advantages and limitations. A field study comparing three commonly-used pore-water sampling techniques (multilevel mini-piezometers, miniature drivepoint samplers and diffusive equilibrium in thin-film gels) was conducted to assess differences in observed nitrate and ammonium concentration profiles. Pore-water nitrate concentrations did not differ significantly between sampling methods (p-value = 0.54) with mean concentrations of 2.53, 4.08 and 4.02 mg l^{-1} observed with the multilevel mini-piezometers, miniature drivepoint samplers and diffusive equilibrium in thin-film gel samplers, respectively. Pore-water ammonium concentrations, however, were significantly higher in pore-water extracted by multilevel mini-piezometers (3.83 mg l^{-1}) and significantly lower where sampled with miniature drivepoint samplers (1.05 mg l^{-1}, p-values <0.01). Differences in observed pore-water ammonium concentration profiles between active (suction: multilevel mini-piezometers) and passive (equilibrium; diffusive equilibrium in thin-film gels) samplers were further explored under laboratory conditions. Measured pore-water ammonium concentrations were significantly greater when sampled by diffusive equilibrium in thin-film gels than with multilevel mini-piezometers (all p-values ≤0.02).

The findings of this study have critical implications for the interpretation of field-based research on hyporheic zone biogeochemical cycling and highlight the need for more systematic testing of sampling protocols. For the first time, the impact of different active and passive pore-water sampling methods is addressed systematically here, highlighting to what degree the choice of pore-water sampling methods affects research outcomes, with relevance for the interpretation of previously published work as well as future studies.

Contents

1. Introduction

Ecohydrological and biogeochemical processes in streambed environments have recently received increasing attention by the hyporheic research community, regulators, policy makers, restoration organisations and utility companies (Boano et al., 2014; Harvey and Gooseff, 2015; Krause et al., 2011a; Krause et al., 2014). This is due in part to the observation of 'hotspots' and 'hot moments' of biogeochemical reactivity in the hyporheic zone (HZ), where surface water and groundwater mix (Krause et al., 2011a; Krause et al., 2017; Lautz and Fanelli, 2008; McClain et al., 2003; Ward, 2016). 'Hotspots' are zones of increased biogeochemical reactivity whereas 'hot moments' are temporal periods of increased biogeochemical reactivity (McClain et al., 2003). These functions arise because hyporheic zones are characterised by high rates of microbial activity, enhanced nutrient cycling and steep redox gradients relative to surface water, leading to descriptions of HZ's and riparian corridors as the "river's livers" (Boulton et al., 1998; Brunke and Gonser, 1997; Fischer et al., 2005; Harvey et al., 2013; Harvey and Gooseff, 2015; Pinay et al., 2018).

Nitrogen is a globally important element that has been affected by anthropogenic activity leading to large inputs of fertiliser into streams and rivers, which negatively impact ecosystem health (Krause et al., 2009; Pinay et al., 2015, 2018; Smith et al., 1999). The high reactivity of the HZ reduces these negative impacts by enhancing nutrient attenuation (Duff et al., 2008; Trimmer et al., 2012). During this process, however, the greenhouse gas N_2O may be produced (Lansdown et al., 2012; Lansdown et al., 2015; Quick et al., 2016). Improved understanding of the nitrogen cycle in these environments, therefore, has large implications for improving water quality and climate change mitigation strategies.

The investigation of streambed biogeochemical processes relies upon the extraction and analysis of interstitial pore-waters, often over

multiple depths and horizontal patterns and over varying timescales. However, despite the growing volume of interdisciplinary research in the HZ, there remains a lack of systematic protocols for sampling methodologies to facilitate transferability between studies (Krause et al., 2011a; Ward, 2016). Sampling, as well as data interpretation, therefore, can be challenging (Kalbus et al., 2006; Rivett et al., 2008). Current sampling techniques have had varying success with capturing nutrient conditions adequately across the respectively relevant spatial and temporal scales (Boano et al., 2014; Krause et al., 2011a), ranging from short-term (minutes to hours) and small-scale (mm-m) to intermediate-term (up to several years) and medium-scale (up to several km). As a result, selecting a pore-water sampling methodology remains non-standard and likely relies on the experience of the practitioner rather than systematic selection that is well-matched to study objectives.

Several pore-water sampling methodologies have been developed over the last couple of decades to best address application-specific challenges in identifying spatial patterns and temporal dynamics of streambed biogeochemical processes. In consequence, we now have at our disposal a wide range of different pore-water sampling tools and methodologies, with variations of how these methods are deployed and applied in practice. Depending on the application, the chosen methods may be based on permanent (e.g. piezometers) (Lee and Cherry, 1979; Rivett et al., 2008) or temporary (e.g. U.S. Geological Survey (USGS) Minipoint samplers, Minipoints from here onwards) (Duff et al., 1998; Harvey and Fuller, 1998) installations (Fig. 1). Although some samplers can extend several metres in depth the majority of sampling techniques developed for extracting pore-water samples for biogeochemical analysis predominantly focus on the upper metre of the streambed, often targeting the top 0.2 m at a higher spatial resolution (Berg and McGlathery, 2000; Duff et al., 1998; Harvey and Fuller, 1998; Krom et al., 1994; Rivett et al., 2008; Sanders and Trimmer, 2006), with the desired vertical scale achievable depending heavily on the technique used, and the volume and rate of pore-water extraction. There are various technical differences between the most commonly used pore-water sampling methods, with respect to their spatial and temporal resolution, sampling volume and rates (few millilitres to several litres) (Bou and Rouch, 1967; Conant Jr. et al., 2004; Duff et al., 1998; Hunt and Stanley, 2000; Kalbus et al., 2006; Krause et al., 2013; Palmer et al., 2007; Rivett et al., 2008), maximum sampling depths (mm's to 2 m) and sampling intervals (Bou and Rouch, 1967; Duff et al., 1998; Hunt and Stanley, 2000; Krause et al., 2011a; Krom et al., 1994; Metzger et al., 2016; Palmer et al., 2007; Rivett et al., 2008; Sanders and Trimmer, 2006).

Each sampling technique may be better suited for different sampling conditions. The ease of installation of samplers in soft, sandy or silty sediments results in these streambeds being the easiest to sample (Dahm et al., 2007). Although gravel and clay sediments provide challenges to sampler installation, both single-depth and multilevel mini-piezometers can be deployed after hammering or pre-drilling (Baxter et al., 2003; Geist et al., 1998; Grimm et al., 2007). Miniature drivepoint samplers are less suitable for gravel, cobble and clay-rich sediments but have been successfully deployed in coarse sediments (Harvey et al., 2013; Ruhala et al., 2018), and although Diffusive Equilibrium in Thin-film (DET) gels are less suitable in gravel sediments, a device for their use in armoured streambeds has been developed (Ullah et al., 2012). If river flow is too high then the use of DET gels may not be appropriate and single-depth piezometers made of rigid pipes may become dislodged during storms (Rivett et al., 2008). The temporary nature of miniature drivepoint sampler installation may also limit their use as they may be easily disturbed.Pore-water sampling methods may be active, requiring pore-water samples to be withdrawn through actively applying pressure by suction via a syringe or pumping (e.g. piezometers), or passive through diffusion where solutes are sampled without actual pore-water extraction but rather through the transfer of solutes into the respective sampler (e.g. DET gels or dialysis membranes), which may influence the sampling outcomes.

Streambed sediments contain pores of varying sizes and connectivity, resulting in different pore-water residence times, redox conditions and nutrient concentrations (Briggs et al., 2014, 2015; Harvey, 1993; Harvey et al., 1995). Active samplers tend to preferentially sample from macropores as the zone of sediment sampled ranges from the largest pores to those of the size related to the applied pressure (Harvey and Gorelick, 1995; Harvey, 1993; Harvey et al., 1995). In contrast, passive samplers preferentially sample micropores or matrix pores (Harvey, 1993; Harvey et al., 1995) as they do not rely on extraction of mobile pore-waters. The mechanical difference between active and passive sampling may have a large effect on nutrient concentrations in the obtained samples. Additionally, the sampling duration can vary between sampling methodologies, with active samplers typically representing a snapshot in time, whereas passive equilibrium samplers represent an integration over the time of diffusive equilibrium (Berg and McGlathery, 2000; William Davison et al., 1994; González-Pinzón et al., 2015). If slow pumping is used with an active sampler, however, this can result in an integrated signal over a similar time period to passive techniques. There are, therefore, substantial differences between sampling techniques. How these differences affect resulting nutrient concentrations remains insufficiently understood.

Here, this work aims to ascertain whether there are differences in the results obtained between different pore-water sampling methodologies to enable researchers to easily select the most appropriate technique and to enable cross-study comparisons of biogeochemical processes in streambed environments. There are three main objectives to meeting this aim: 1) to provide technical information on pore-water sampling techniques to aid in sampler selection, 2) to investigate the differences in pore-water nutrient profiles and subsequent streambed characterisation obtained from three common pore-water sampling methodologies and 3) to investigate differences in porewater ammonium (NH_4^+) profiles from the use of active versus passive samplers.

A literature review of the most common pore-water sampling techniques, discussing their specific advantages and limitations for specific applications is presented. Subsequently, the outcomes of a selection of common pore-water sampling methodologies were compared in a comparative in-situ field study, assessing the ability of multilevel mini-piezometers and Minipoints (as examples of active samplers), and DET gel probes (as examples of passive samplers) (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012) to capture nutrient patterns in streambed pore-waters across a stream reach at varying spatial resolutions. These methods all allow pore-water nutrient concentrations to be determined at multiple depths within the streambed and cover a variety of spatial resolutions and both active and passive sampling. The more common multilevel mini-piezometer setup, with a coarser resolution and a greater depth range than Minipoints and DET gels, was used here to provide comparison with other techniques as they are widely applied in field-based research. Data were, therefore, compared within the top 0.15 m of the streambed, where the sampling zones of all three techniques overlap. A laboratory control experiment comparing NH_4^+ pore-water concentrations gained from multilevel mini-piezometers and DET gels was conducted to determine whether differences observed in the in-situ study were due to sampler differences or field-specific conditions.

2. Literature review: comparison of sampling techniques

Various literature reviews have previously provided comparative analyses of the performance of experimental methods for streambed characterisation; however, these have either predominantly focussed on methodologies to determine hydrological properties of streambeds or on only active or passive sampling (e.g. Davison et al., 2000; González-Pinzón et al., 2015; Kalbus et al., 2006; Landon et al., 2001; Scanlon et al., 2002). This study focusses on the comparison of streambed sampling methodologies developed to analyse vertical profiles of

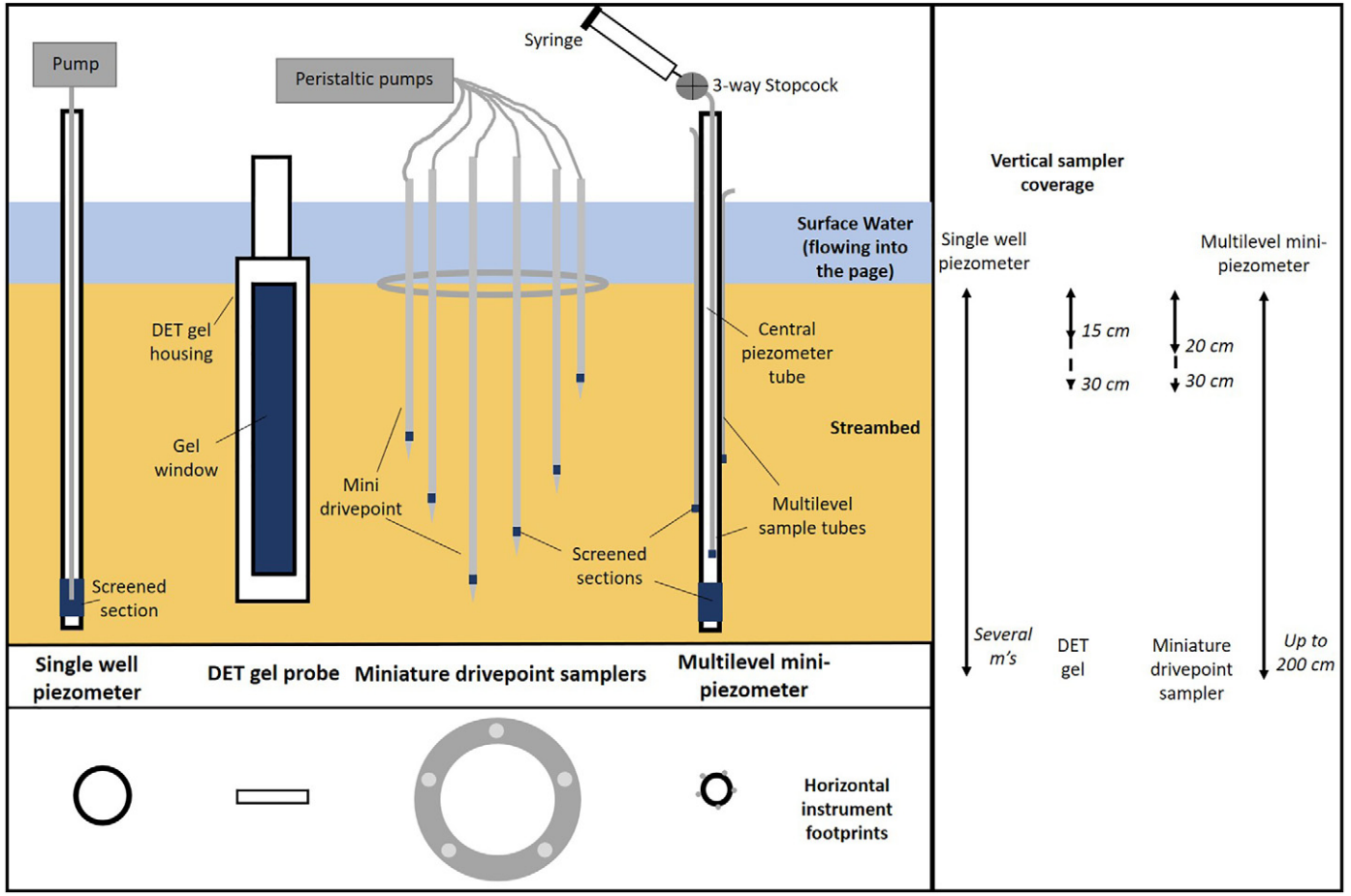

Fig. 1. Conceptual diagram of main streambed pore-water sampling techniques for analysis of biogeochemical cycling in hyporheic zones, including (from left to right): single well piezometers, diffusive equilibrium in thin-film (DET) gels, (Davison et al., 1991; Harper et al., 1997) miniature drivepoint samplers (example shown: USGS Minipoint sampler; Duff et al., 1998; Harvey and Fuller, 1998), and multilevel mini-piezometers. Also shown (on the right) are the vertical ranges covered and horizontal instrument footprints of the respective pore-water sampling techniques.

Table 1
Comparison of key characteristics, advantages and limitations of most frequently used streambed pore-water sampling methodologies identified during the literature review discussed in text.

Sampling methodology	Active or passive sampling	Sampling technique	Sampling depth	Horizontal instrument footprint	Vertical resolution	Temporal resolution	Deployment time	Advantages	Limitations
Single-depth piezometers	Active	Porewater extraction	Up to several m's	10–50 mm	> 100 mm's	Snapshot during time of sampling	Days to years	- Hydrological information at location of chemical sampling - Large sample volume - Easy installation in sandy and silt sediments - Permanent logger installation	- Must be installed prior to sampling (hours to days before) - Substantial hammering or pre-drilling is required in gravel and clay sediments - Time to refill after purging can be long, preventing sampling or exposing sample to the atmosphere - Large horizontal instrument footprint - Low vertical resolution - Although hyporheic fluxes can be estimated, this assumes vertical flow is present, which is not always the case - The larger piezometer design may alter hyporheic flow
Multilevel mini-piezometers	Active	Porewater extraction	0.1 to 2 m	30 mm	50–100 mm	Snapshot during time of sampling	Days to years	- Hydrological information obtained in central tube - Hyporheic fluxes and reaction rates can be determined at all depths - Porewater extraction from discrete, user-defined depths - Easy installation in soft sediments - Small sampling diameter due to small horizontal instrument footprint - Flexible, more storm-resilient piezometer, less prone to vandalism - Sampling via a closed loop when syringes are used	- Hydrological information gained via hydraulic gradients is not possible to determine in the multilevel sampling tubes, so information is only attainable from the depth of the central piezometer - The central piezometer is typically too small for permanent loggers - The vertical solute profile may be disrupted if sampling occurs too rapidly - Coarse sampling interval - Installment a few days prior to sampling is required - Installation is difficult in gravel or clay sediments, and may require substantial pre-drilling or hammering - Although hyporheic fluxes can be estimated, this assumes vertical flow is present, which is not always the case
Miniature drivepoint samplers	Active	Porewater extraction	up to 0.4 m	50–100 mm	10–30 mm	Snapshot during time of sampling	Hours to days	- Hydrological information and reaction rates can be determined at all depths - Small diameter allows easy and rapid installation with minimal disturbance, allowing use as roaming samplers and to sample unstable sediments - Porewater samples can be pre-filtered at the sampler tip or in line during pumping - High resolution porewater extraction	- The temporary nature of installation prevents longer temporal studies in the same location and the samplers may be easily disturbed - Installation success may be affected by sediment type, gravel, cobble or clay-rich can be problematic - The horizontal instrument footprint is relatively large, resulting in lateral spacing of the vertical solute profiles - The vertical solute profile may be disrupted if sampling does not occur at low flow rates - The screening or filter at the base of the drivepoint is prone to clogging

(continued on next page)

Table 1 (*continued*)

Sampling methodology	Active or passive sampling	Sampling technique	Sampling depth	Horizontal instrument footprint	Vertical resolution	Temporal resolution	Deployment time	Advantages	Limitations
Diffusive equilibrium in thin-film (DET) gels	Passive	Solute equilibration	0.15–0.3 m	18–20 mm	10 mm (1 mm is theoretically possible)	Integrated over time of diffusive equilibration	At least 72 h	- The nature of passive sampling prevents disturbance of the vertical solute profile as long as diffusion within the gel is minimal - Quick and easy installation in soft sediments - High vertical resolution - Small horizontal instrument footprint	- Information on hydraulic gradients cannot be determined from these samplers - Although hyporheic fluxes can be estimated, this assumes vertical flow is present, which is not always the case - Installation is difficult in gravel sediments - No hydrological information can be determined from the DET gel - The gel requires installation ahead of sampling (72 h has been suggested) - Vertical diffusion may occur within the gel, which can reduce profile fidelity, both during deployment and after removal - The 40 mm wide plastic frame may alter hyporheic flow

nutrients, which enable ecohydrological investigations across surface water-groundwater interfaces. A summary of the following literature review can be found in Table 1.

2.1. Active samplers

2.1.1. Single-depth piezometers and single-depth mini-piezometers

Single-depth piezometers are used to sample pore-water at depths of up to several metres and are typically constructed from a steel, polyvinyl chloride (PVC) or high-density polyethylene (HDPE) pipe, which is screened at the bottom end over the desired vertical range; the bottom of the pipe is then blocked (Fig. 1) (Argerich et al., 2011; Baxter et al., 2003; Conant Jr. et al., 2004; Dahm et al., 2007; Geist et al., 1998; Grimm et al., 2007; Lee and Cherry, 1979; Lewandowski et al., 2015; Rivett et al., 2008). A screened section varying between tens and hundreds of millimetres is utilised depending on whether depth-specific or depth-integrated sampling is required (Baxter et al., 2003; Dahm et al., 2007; Geist et al., 1998; Winter et al., 1998). An alternative design, using porous (20 μm mean pore diameter) HDPE pipe, which does not require a screened section has also been used (Wondzell and Swanson, 1996). While piezometers sample water at a single depth, multiple piezometers may be nested to allow sampling at multiple depths, covering a larger horizontal instrument footprint, which are typically sampled consecutively (Battin et al., 2003a; Baxter et al., 2003; Käser et al., 2009; Krause et al., 2009). The instrument footprint of a single piezometer is typically 10–50 mm in diameter (Argerich et al., 2011; Baxter et al., 2003; Blume et al., 2013; Conant Jr. et al., 2004; Dahm et al., 2007; Geist et al., 1998; Krause et al., 2009; Rivett et al., 2008; Valett et al., 1994; Wondzell and Swanson, 1996), which can result in a relatively large instrument footprint when a nested design is utilised. Piezometers are deployed in the streambed usually for longer time scales of several weeks to years (Argerich et al., 2011; Dahm et al., 2007; Lee and Cherry, 1979), and the extracted pore-water sample represents a snapshot of the conditions at the time of sampling (González-Pinzón et al., 2015). Prior to sampling, piezometers have to be purged of water by pumping until dry or until multiple times the water volume has been removed if complete purging is not feasible (Johnson et al., 2004; Krause et al., 2009; Lapworth et al., 2009). Pore-water is sampled from the piezometer with a pump or syringe once it has refilled, hence, the pore-water is not extracted through suction from the sediment, but through ambient pore-water flow into the piezometer (Dahm et al., 2007), and is, therefore, affected by the hydrological conditions of the stream, i.e. gaining or losing and surface water level.

2.1.1.1. Advantages. Information on exchange fluxes between stream and subsurface, and properties such as hydraulic gradients and hydraulic conductivity can be obtained in the piezometer at the depth of sampling (Argerich et al., 2011; Baxter et al., 2003; Dahm et al., 2007; Datry et al., 2015; González-Pinzón et al., 2015; Grimm et al., 2007; Kalbus et al., 2006; Lee and Cherry, 1979; Valett et al., 1994), allowing hydrological and chemical information to be gained at the same location and through the same sampling device. The wide diameter of the piezometer also enables permanent installation of loggers to measure a variety of parameters including temperature, electrical conductivity, turbidity and pressure. The design, with water flowing into the piezometer (Dahm et al., 2007), allows larger volumes of water to be extracted than is attainable with other sampling methods. Furthermore, piezometer installation is straightforward in sandy and silt sediments, and if a wider spatially-integrated signal is required, the relatively large sampling footprint may be advantageous.

2.1.1.2. Limitations. Single-depth piezometers must be installed with sufficient time prior to sampling for the natural conditions of the streambed to re-establish, this time can be long (hours to days), especially when installing into clay, silt or shale sediment (Lewandowski et al., 2015; Ohio EPA, 2012). Piezometer installation in gravel and clay sediments can be difficult, and requires substantial hammering or pre-drilling of the sediment (Baxter et al., 2003; Geist et al., 1998; Grimm et al., 2007). The time taken for the piezometer to refill after purging can be long, in some cases prohibiting sampling, exposing pore-water to exchange with the atmosphere affecting dissolved gases. Additionally, the horizontal instrument footprint of the piezometer is relatively large, and the achievable vertical resolution is low compared to other techniques. Although hyporheic pore-water fluxes can be estimated, this assumes vertical flow is present, which is not always the case

(González-Pinzón et al., 2015), and reaction rates cannot be determined with this technique. Additionally, if the larger piezometer design is used (up to ~ 50 mm) this may alter the hyporheic flow at the sampling location (Ward et al., 2011).

2.1.2. Multilevel mini-piezometers

Multilevel mini-piezometers consist of a number of small Tygon© or PTFE tubes of different lengths, which are fitted around a larger diameter central steel, PVC or HDPE tube (acting as a more traditional piezometer, Fig. 1) (Krause et al., 2013; Lewandowski et al., 2011, 2015; Rivett et al., 2008; Shelley et al., 2017). The piezometer design allows the extraction of pore-water at multiple discrete sampling depths and intervals, with minimal lateral spacing, which are defined by the user (Rivett et al., 2008). Sampling depths are typically between 0.1 and 2 m (Gooddy et al., 2014; Heppell et al., 2013; Krause et al., 2011b; Krause et al., 2013; Lansdown et al., 2015; Rivett et al., 2008; Shelley et al., 2017), with a vertical sampling interval of 0.1 m (Lansdown et al., 2015; Rivett et al., 2008; Shelley et al., 2017), although a vertical spatial resolution up to 50 mm is achievable with a low pore-water extraction rate (Rivett et al., 2008). The horizontal instrument footprint of the multilevel mini-piezometer setup is small, usually ~30 mm in diameter due to a relatively small diameter central piezometer tube, allowing depth profiles to be sampled over a small horizontal area of the streambed (Krause et al., 2013; Rivett et al., 2008; Shelley et al., 2017). Multilevel mini-piezometers are deployed into the streambed to usually remain for time periods between several days to years (Rivett et al., 2008), and the extracted pore-water sample represents a snapshot of the conditions at the time of sampling. Sample volumes are typically small and collected slowly with a syringe or with a peristaltic pump at a low flow rate, which limits disturbance to the hyporheic flow, as well as allowing a higher vertical resolution to be achieved (Krause et al., 2013; Lewandowski et al., 2015). If low pumping rates are used then the time taken for sampling may integrate a changing nutrient signal if sampling under rapidly changing environmental conditions. The multiple depths of the multilevel mini-piezometers may be sampled simultaneously or consecutively. A pore-water sampler combining attributes of the single-depth piezometer and the multilevel mini-piezometers has recently been developed, using a relatively large central piezometer (32 mm outer diameter) up to 4 m depth (Gassen et al., 2017). Sampling ports are connected to the central tube so that the sampling resolution varies from 0.05 to 0.5 m, depending on which zone is being sampled at that depth. Although this affords high-resolution sampling at critical zones with a large depth profile, this sampling methodology retains the issues associated with a large horizontal instrument footprint.

2.1.2.1. Advantages. Hydraulic gradient, hydraulic conductivity and hyporheic exchange can be determined in the central piezometer tube provided its internal diameter is large enough to be manually dip-metred (Baxter et al., 2003; Dahm et al., 2007; Grimm et al., 2007; Lee and Cherry, 1979), while residence times and hyporheic water fluxes may be determined in the multilevel tubes, therefore, reaction rates can also be calculated using this technique (Shelley et al., 2017). Multilevel mini-piezometers allow pore-water samples to be extracted from discrete depths, enabling vertical solute profiles to be captured (Krause et al., 2013; Rivett et al., 2008). Their design, which is both compact and user-defined, leads to easy installation in soft sediment (Dahm et al., 2007) and a small sampling diameter (Krause et al., 2013; Rivett et al., 2008; Shelley et al., 2017), as well as a flexible vertical depth and resolution (Rivett et al., 2008), to target focus areas based on the specific research questions. The central piezometer tube is flexible and so bends with surface water flow resulting in a more storm-resilient piezometer, less likely to be displaced or contaminated during storms, than more traditional, rigid single-well piezometers (Rivett et al., 2008). The flexible design also causes less visual disturbance; therefore, these samplers are also less prone to vandalism. Furthermore, the larger range of sampling available when using multilevel mini-piezometers allows streambed biogeochemistry to be investigated at a higher spatial (vertical) resolution and depth. Sampling with syringes or pumping into syringes prevents contact with the atmosphere eliminating issues of exchange of dissolved gases.

2.1.2.2. Limitations. The hydrological information gained via hydraulic gradients is difficult to determine in the discrete depths of the multilevel mini-piezometers, due to the small diameter of the multilevel sampling tubes (Rivett et al., 2008). Only the central piezometer tube, therefore, can provide information on hydraulic gradients (Krause et al., 2013; Rivett et al., 2008). Hence, it is not possible to ascertain this information for each sampling depth and only information at the deepest location of the piezometer is available. Additionally, the central tube is usually too small to allow installation of continuous monitoring devices for hydraulic heads, electrical conductivity, turbidity or different solute chemical parameters. There is a risk of disrupting the vertical solute profile during sampling, as drawing samples at too high flow rate or at too great a vacuum may cause overlap in the sample area between depths or alter preferential flow (artificially increasing horizontal or vertical flow) in the streambed (Krause et al., 2013). The sampling interval achievable using multilevel mini-piezometers is relatively coarse (typically 50–100 mm's) compared to other discrete depth-sampling techniques (Berg and McGlathery, 2000; Duff et al., 1998; Harvey et al., 2013; Rivett et al., 2008; Sanders and Trimmer, 2006). The piezometers are usually installed several days in advance of sampling to allow the sediment to re-settle around the piezometer and for the ambient flow conditions to re-establish (Lewandowski et al., 2015). In gravel or clay sediments, installation can be more difficult and may require pre-drilling of a hole or substantial hammering to install the piezometer into the streambed (Baxter et al., 2003; Grimm et al., 2007). Although hyporheic fluxes can be estimated, this assumes vertical flow is present, which is not always the case (González-Pinzón et al., 2015).

2.1.3. Miniature drivepoint samplers

Miniature drivepoints have been developed to sample streambed chemistry at high vertical resolution with minimal disturbance caused at the streambed (Berg and McGlathery, 2000; Duff et al., 1998; Harvey and Fuller, 1998; Sanders and Trimmer, 2006). Several variations and design adaptations have been developed over time, including: 1) six ~3 mm diameter, stainless steel drivepoints fixed in a 0.1 m diameter circle on a plastic disk (USGS Minipoint sampler, shown as example in Fig. 1) (Duff et al., 1998; Harvey and Fuller, 1998), 2) nine 8 mm diameter drivepoints held in a PVC or stainless steel ring (Sanders and Trimmer, 2006) and 3) a single 2.4 mm diameter, stainless steel drivepoint, which is deployed successively for spot sampling through six guiding holes in a 47 mm diameter circle on an acrylic plate (Berg and McGlathery, 2000).

Water is sampled through a screened section near the tip of the drivepoint, which typically comprises of slots (Duff et al., 1998; Harvey and Fuller, 1998) or holes (Berg and McGlathery, 2000; Sanders and Trimmer, 2006). The drivepoint samplers are installed to discrete, user-defined depths to enable the upper 0.4 m of the streambed to be sampled at high vertical resolution, between 10 and 30 mm (Berg and McGlathery, 2000; Duff et al., 1998; Harvey et al., 2013; Harvey and Fuller, 1998; Sanders and Trimmer, 2006). The horizontal instrument footprints of miniature drivepoint samplers are relatively large resulting in pore-water samples collected from different depths over a wider area than those from a multilevel mini-piezometer. These samplers are usually installed shortly before sampling, enabling them to be used as roaming samplers, with extracted samples representing a snapshot of the conditions at the time of sampling (González-Pinzón et al., 2015; Sanders and Trimmer, 2006). Due to the usually low pumping rates used for sampling, however, this sampling time can be long. Samples collected using miniature drivepoint

samplers tend to be of relatively small volume (1.5–70 ml) (Berg and McGlathery, 2000; Duff et al., 1998; Harvey and Fuller, 1998; Sanders and Trimmer, 2006) and are extracted slowly using a syringe or a peristaltic pump with very low flow rates (Berg and McGlathery, 2000; Duff et al., 1998; Harvey and Fuller, 1998). This prevents the ambient hyporheic flow from being disturbed, as well as maintaining a high vertical resolution (Duff et al., 1998; Harvey and Fuller, 1998). The discrete sampling depths may be sampled simultaneously (Duff et al., 1998; Harvey et al., 2013; Harvey and Fuller, 1998) or consecutively. Sampling with syringes or pumping into syringes prevents contact with the atmosphere eliminating issues of exchange of dissolved gases.

2.1.3.1. Advantages. Residence times, hyporheic fluxes and hyporheic exchange can be determined at multiple depths using miniature drivepoint samplers (González-Pinzón et al., 2015), providing measurements that allow calculation of reaction rates (Harvey et al., 2013; Knapp et al., 2017). The combination of small sample volumes and low extraction rates enables sampling with minimal disturbance to the ambient hyporheic flow, allowing high-resolution pore-water extraction, which is difficult to achieve with other piezometer methods (Harvey and Fuller, 1998). The small diameter of miniature drivepoint samplers enables easy and rapid installation with minimal disturbance to the streambed (Berg and McGlathery, 2000; Duff et al., 1998; Harvey and Fuller, 1998; Sanders and Trimmer, 2006). This allows the drivepoints to be sampled shortly after deployment and used effectively as roaming samplers where probes are installed, sampled and then removed, before installation at a new location. The short deployment time also enables unstable and unconsolidated sediments, which may move frequently between events, to be sampled. Pore-water samples can be pre-filtered at the tip of the probe through its design (Berg and McGlathery, 2000) or glass wool (Sanders and Trimmer, 2006), or filtered in-line during pumping (Harvey et al., 2013).

2.1.3.2. Limitations. Given the temporary nature of the installation of miniature drivepoint samplers, they cannot be installed for long periods and so longer temporal studies would not be conducted in exactly the same location. Additionally, their ease of deployment and removal for roaming surveys means these samplers may be more easily disturbed than permanent installations, and so the depth of sampling could be compromised. The success of miniature drivepoint sampler installation can be heavily dependent on sediment type as deployment in gravel, cobble or clay-rich sediments is challenging (Ruhala et al., 2018), despite this, samplers have been successfully used in coarse sediments (Harvey et al., 2013). The relatively large horizontal instrument footprint (Berg and McGlathery, 2000; Duff et al., 1998; Sanders and Trimmer, 2006), resulting in samples from different depths not being vertically aligned where drivepoints are held in sampling arrays as is the designs of many drivepoints, may result in inaccurate vertical profiles where small-scale heterogeneity in sediment properties occurs. Pore-water samples must be extracted from miniature drivepoint samplers at a low rate to prevent pore-water being drawn from outside of the intended sampling depth, and to prevent changes in preferential flow, to preserve the high spatial resolution (Berg and McGlathery, 2000; Harvey et al., 2013; Harvey and Fuller, 1998; Sanders and Trimmer, 2006). The screening or filter at the base of miniature drivepoint samplers is prone to clogging in silt, clay or organic-rich sediments, which may disrupt sampling and reduce the lifetime of the filter (which tends to be difficult to change) if one is used with the drivepoint design. It is not possible to determine information on hydraulic gradients from these samplers due to the small inner diameter of sampling tubes. Hyporheic fluxes can be estimated under the assumption that vertical flow is present, which is not always the case (González-Pinzón et al., 2015).

2.2. Passive equilibration samplers

2.2.1. DET gel probes

DET gel probes (Davison et al., 1991; Harper et al., 1997) are passive samplers consisting of a polyacrylamide hydrogel (Davison et al., 1994; Krom et al., 1994; Mortimer et al., 1998; Ullah et al., 2012), which contains ~95% water, is between ~0.4 to 1.8 mm thick, and housed in a plastic probe (Davison et al., 1991; Harper et al., 1997; Krom et al., 1994; Ullah et al., 2012). DET gels are available in either $NaNO_3$ or NaCl buffer, with the buffer dependent on the type of solutes to be analysed (DGT Research Ltd.; www.dgtresearch.com). Rather than extracting pore-water actively from the streambed, solutes in the investigated substrate diffuse across the DET gel membrane, into and out of the gel, until equilibrium with the pore-water is reached (Davison and Zhang, 1994; Davison et al., 1991; Davison et al., 1994; Harper et al., 1997). The gel probes are then removed from the sediment, the gel sliced at the required vertical resolution, and back-equilibrated with a known volume of ultrapure water (Krom et al., 1994; Mortimer et al., 1998). The concentration of solute in the DET gel slices and hence, the pore-water is determined from this eluate (Harper et al., 1997).

Commercially available DET gels are typically 0.15 m in length and so this vertical range is usually sampled, however, they have also been modified and used for streambed pore-water sampling at depths up to 0.3 m (Fig. 1) (Ullah et al., 2012). The vertical resolution attained by the DET gel is determined by the interval at which the gel is either partitioned within the probe or immediately sliced at upon removal from the sediment (Davison et al., 1994; Mortimer et al., 1998). Vertical sampling resolutions in the mm range are possible if slicing occurs fast enough after removal to avoid vertical diffusion within the gel or if the DET gel is constrained at the desired resolution (Dočekalová et al., 2002; Harper et al., 1997; Krause et al., 2013; Krom et al., 1994; Ullah et al., 2012). Recently, DET gels have been combined with colorimetry and hyperspectral imagery, which enables two-dimensional nitrite and nitrate (NO_3^-) distributions to be simultaneously measured at millimetre scale (Metzger et al., 2016). The horizontal instrument footprint of the DET gel probe is ~5 mm × 40 mm, however, the exposed membrane of the gel is only 18–20 mm wide (Krause et al., 2013; Krom et al., 1994; Mortimer et al., 1998). DET gel probes are usually deployed into the sediment for at least 72 h prior to retrieval to allow ambient flow conditions to re-establish after installation and equilibrium with the pore-water to be reached (Byrne et al., 2015; Mortimer et al., 1998; Ullah et al., 2012). Due to the DET gel being an equilibration technique the samples collected represent an average of the biogeochemical concentrations dynamics over the time of diffusive equilibration within the sediment, i.e. the time for solute concentrations to equilibrate between pore-water and gel rather than deployment time (Berg and McGlathery, 2000; Davison et al., 1994). The nature of this technique means that all depths are sampled simultaneously and environments which are diffusion-dominated with low solute velocities are most suitable for sampling with diffusion equilibrators (Duff et al., 1998).

2.2.1.1. Advantages. The passive sampling of solutes through diffusion into the sampler prevents potential issues associated with streambed pore-water extraction preventing crossover between depths as long as diffusion within the gel is minimum (Dočekalová et al., 2002; Harper et al., 1997). Installation in soft sediment is quick and easy, requiring only pushing into the sediment by hand. The DET gel sampler has a very high vertical resolution (Harper et al., 1997; Krom et al., 1994; Ullah et al., 2012), and the horizontal instrument footprint is small minimising the lateral distribution of the vertical profile (Krause et al., 2013; Krom et al., 1994; Mortimer et al., 1998). Despite the potential for the highest spatial resolutions of all analysed methods any biogeochemical patterns lesser or equal to the gel slicing resolution cannot be resolved (Harper et al., 1997).

2.2.1.2. Limitations. Difficulty can arise in deployment of DET gel probes in gravel sediments, although Ullah et al. (2012) developed a stainless-steel installation device and successfully deployed the DET gel probes in an armoured gravel bed. As the DET gel probe is not a piezometer, no hydrological information, such as hydraulic gradients or hyporheic flow, can be ascertained from the device, therefore, information is limited to pore-water solute concentrations. The long time required for DET gel deployment prior to sampling requires careful planning (Mortimer et al., 1998; Ullah et al., 2012). Furthermore, the vertical resolution may be compromised by vertical diffusion within the DET gel, which is dependent on gel thickness and time between removal and slicing (Davison et al., 1994; Harper et al., 1997). The 40 mm wide plastic frame of the gel bears the risk of altering the hyporheic flow at the sampling location (Ward et al., 2011).

3. Comparative study of sampling methodologies

The literature review indicated key differences between the common streambed sampling technologies available, most notably in sampling technique (active versus passive), spatial and temporal resolution, and sampling range. Here we explore these differences through a comparative experimental analysis using some of the most frequently used sampling methodologies with important differences. These methodologies include active and passive sampling techniques and span a range of vertical resolutions and sampling scales.

3.1. Method comparison experiment

3.1.1. In-situ experiment

An in-situ field study was performed to compare the impact of applied pore-water sampling methods on observed streambed nutrient patterns, using multilevel mini-piezometers and Minipoints (as examples of active samplers), and DET gel probes (as examples of passive samplers) (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012).

3.1.1.1. Study site. The study was conducted in the Hammer stream in West Sussex, UK (Fig. 2), which is typical of lowland rivers experiencing increased nitrate loading. The Hammer is a sandy stream, which drains a 24.6 km^2 catchment with bedrock predominantly made up of greensands and mudstones (Blaen et al., 2018; Shelley et al., 2017; BGS, 2016). Land-use within the catchment is predominantly agricultural, with smaller patches of deciduous broad-leafed woodland, with the Hammer stream flowing through a deciduous forested valley at the experimental site (Blaen et al., 2018; BGS, 2016), and the mean annual precipitation is 790 mm (UK Met Office, 2016).

The application of the different field sampling methods focussed on an approximately 60 m meandering reach of the stream (Fig. 2), where the streambed was dominated by spatially-homogeneous, sandy sediment (Shelley et al., 2017). The study reach is characterised by multiple bedforms including pools and bars, and has extensive woody debris. Stream discharge at the experimental site typically ranged between 70 and 120 l s^{-1}, however, discharge may exceed 1000 l s^{-1} during storm events that typically occur in winter (Blaen et al., 2018). The river valley is underlain by expansive, low conductivity peat deposits and clay lenses at 1–2 m depth, which inhibit groundwater upwelling, therefore, the regional groundwater contribution is not expected to cause significant inputs (Shelley et al., 2017).

3.1.1.2. Multilevel mini-piezometers. Pore-water samples were collected on the 9th July 2015 from 40 multilevel mini-piezometers (Fig. 2c), installed more than one year in advance of the experiment. Pore-water samples (10 ml) were manually collected from the multilevel mini-piezometers at depths of 0.1, 0.2, 0.3, 0.5 and 1 m using a syringe.

Pore-water samples were immediately filtered (0.45 μm Whatman) into acid-washed (10% HCl) vials, stored cool and in the dark in the field, and frozen once returned to the laboratory until laboratory analysis. Pore-water samples were analysed for nitrate and ammonium concentration using a continuous flow analyser (San++, Skalar, Breda, The Netherlands), with a limit of detection and precision of 0.01 ± 5% mg NH_4^+-N l^{-1} and 0.001 ± 1% mg NO_3^--N l^{-1}.

3.1.1.3. Minipoint samplers. Pore-water samples were collected twice between the 16th and 18th June 2015 from 16 Minipoint samplers (Fig. 2c), installed on the day of sampling. Pore-water samples (50 ml) were slowly pumped from the Minipoint samplers using a multichannel peristaltic pump at depths of 25, 50, 75, 100, 125 and 150 mm. Surface water samples were also taken at this time. Pore-water samples collected from Minipoint samplers were immediately filtered (0.45 μm Whatman) into acid-washed (10% HCl) vials, stored cool and in the dark in the field, and frozen once returned to the laboratory until laboratory analysis. Pore-water samples were analysed for nitrate and ammonium concentration using a continuous flow analyser (San++, Skalar, Breda, The Netherlands). A different Skalar instrument was used for the samples from each method resulting in Minipoint sampler samples analysed with an accuracy and precision of 0.1 and ± 0.02 mg NH_4^+-N l^{-1} and 0.14 and ± 0.01 mg NO_3^--N l^{-1}, respectively, and a limit of detection of 0.02 mg N l^{-1} for ammonium and nitrate, using a 3 mg N l^{-1} standard for both ammonium and nitrate.

3.1.1.4. DET gels. The DET gels were deployed on the 10th and 11th June 2015, so that they were co-located with 21 of the multilevel mini-piezometers. The DET gels were removed on the 17th June 2015 and sliced at 50 mm intervals (ultrapure water-rinsed blade on an acid-washed (10% HCl) board) within 5 min of removal. The DET gel slices were stored in acid-washed (10% HCl) centrifuge tubes at 4 °C until laboratory analysis within four months.

3.1.1.4.1. Elution of DET gels. The gels were weighed to determine the volume of water within the DET gel slice (assumed water content of 95%) and 5 ml of ultrapure (18.2 MΩ) water added to each tube. The gels were back-equilibrated by shaking, on ice, for 20 h, after which, the gels were removed, and the eluate frozen for storage until analysis. Eluate samples were analysed for nitrate and ammonium concentration using a continuous flow analyser (San++, Skalar, Breda, The Netherlands), with an accuracy and precision of 0.1 and ± 0.02 mg NH_4^+-N l^{-1} and 0.14 and ± 0.01 mg NO_3^--N l^{-1}, respectively, and a limit of detection of 0.02 mg N l^{-1} for ammonium and nitrate, using 0.61 and 1.01 mg N l^{-1} standards, respectively. The concentration within the gel, and hence the pore-water, was then calculated using the volume of water within the gel slice.

3.1.2. Laboratory experiment

Fine, sand-dominated stream sediment was collected from the Mill Brook at the Birmingham Institute of Forest Research, Staffordshire, UK in May 2016, see Blaen et al. (2017) for site information. Moist sediment was sieved (16 mm), homogenised and placed into three 10 l containers. Solutions of varying ammonium concentrations (0.0, 4.9 and 10.0 mg NH_4^+ l^{-1}) were made from a stock of NH_4Cl and 10 l of solution was added to each of the three containers resulting in saturated sediment, and DET gels and multilevel mini-piezometers, with sampling depths of 25, 75 and 125 mm, were installed into the sediment. After three days, the DET gels were removed and sliced at 50 mm intervals, and the multilevel mini-piezometers were sampled. Three additional DET gels were equilibrated in ultrapure water (18.2 MΩ) for 24 h for quality control purposes. The DET gels were processed as detailed in Section 3.1.1.4, and all samples were stored frozen until analysis.

3.1.3. Statistical analysis

The nitrate and ammonium data obtained from each technique in the field and laboratory studies were checked for normality and equality of variances, and the appropriate parametric or non-parametric test applied to determine whether differences between methods were significant. In the field study, assessment of any differences (p-value <0.05) in

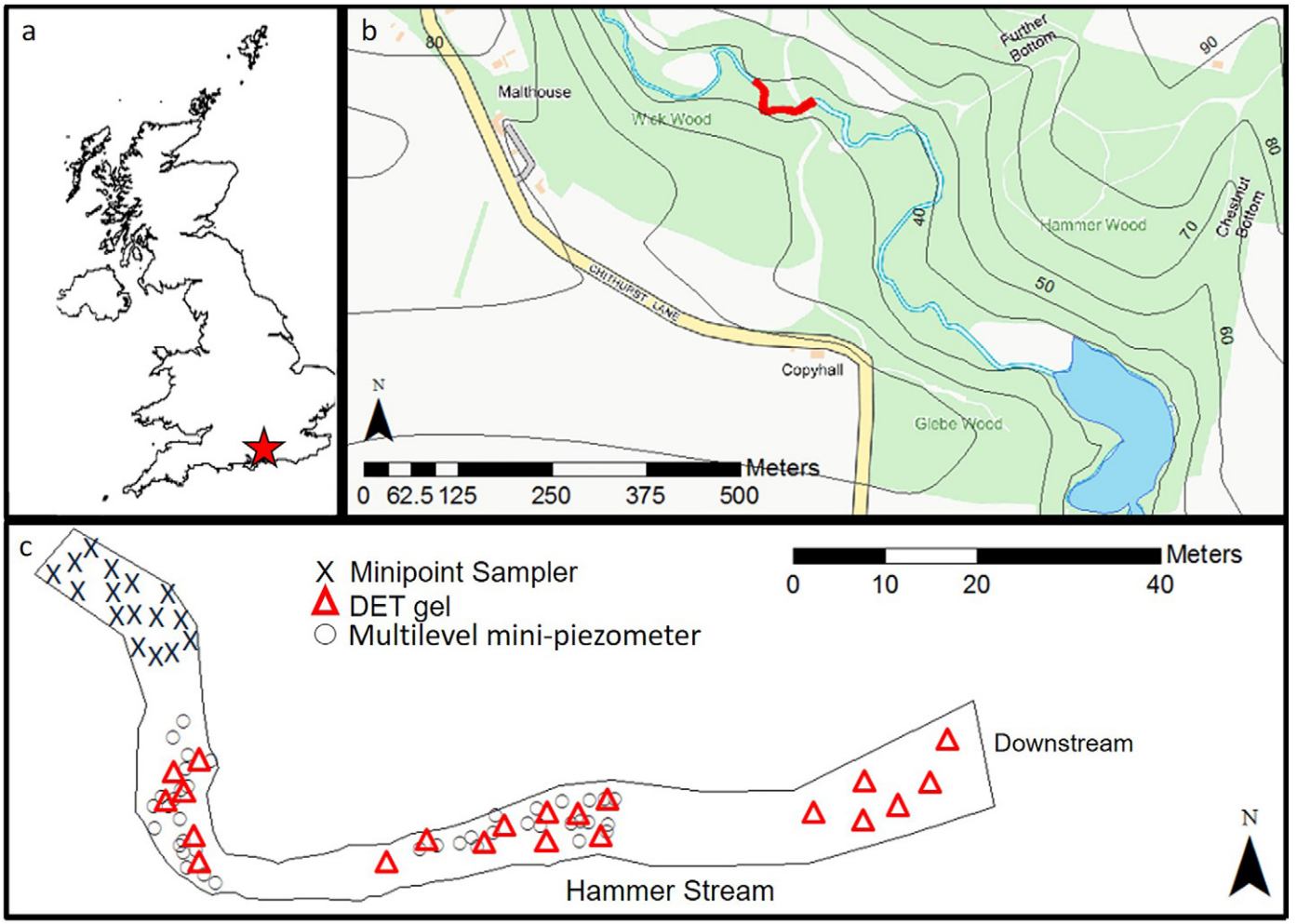

Fig. 2. Location of a. the Hammer stream within the UK (represented by the red star), b. the study reach (indicated by the red section) at the Hammer Stream, green indicates woodland and white indicates agricultural land and c. the location of the different sampling devices used in this study.

measured nitrate and ammonium from the three sampling methods were determined using the non-parametric Kruskal-Wallis rank sum test. If significant differences between the groups were identified, a Dunn test was performed to identify which groups were statistically different. In the laboratory study, significant differences (p-value <0.05) in ammonium between sampling methods were determined using a paired t-test or the equivalent non-parametric Wilcoxon rank sum test.

3.2. Results

3.2.1. Field study

3.2.1.1. Pore-water nitrate

3.2.1.1.1. Vertical concentration profiles in the top 1 m of the streambed. The comparison of the techniques in this section, and all subsequent sections, refers to the precision of the techniques, as the actual pore-water nutrient concentrations are unknown. The nitrate depth profiles observed varied depending upon which sampling technique was used (Fig. 3); the greatest individual porewater nitrate concentrations were observed in the DET gel samples, however, more samples taken with the Minipoints had relatively high concentrations. The concentrations in the multilevel mini-piezometer samples were predominantly lower than those found during sampling with either the DET gels or the Minipoints. Mean pore-water nitrate concentrations were determined at each sampling depth used for each method and were typically highest in the data from the DET gels (3.78 to 4.34 mg l^{-1}), although the highest mean pore-water concentrations in the shallowest depths were found using the Minipoints (10.22 and 5.86 mg l^{-1} at 2.5 and 5 cm, respectively). The largest range of mean pore-water nitrate concentrations per depth was observed in the Minipoint data (9.67 mg l^{-1}, Fig. 4). There was no statistically significant difference (p-value = 0.54, Table 2) in nitrate concentrations between the methods used. The clearest trend in mean pore-water nitrate concentration with depth was observed in the Minipoint data (Fig. 4), where mean pore-water nitrate concentrations decreased non-linearly with depth, from 10.2 to 0.54 mg l^{-1} over a depth interval of 25 to 150 mm below the streambed interface. The small range in mean concentrations per depth captured by the DET gels and multilevel mini-piezometers (3.78 to 4.34 mg l^{-1} and 0.73 to 2.53 mg l^{-1} for DET gels and multilevel mini-piezometer samples, respectively) prevented such a clear trend from being observed, although the vertical concentration profile from the multilevel mini-piezometer data was similar to the one observed in the Minipoints (Fig. 4).

3.2.1.1.2. Vertical concentration profiles in the top 0.15 m of the streambed. Descriptive statistics were calculated individually for each method from all data collected in the top 0.15 m of the streambed as this represents the overlap of the window of detection for the sampling methods. The highest mean pore-water nitrate concentration was observed in the Minipoint samples (4.08 mg l^{-1}) and DET gel samples (4.02 mg l^{-1}), in comparison the mean pore-water nitrate concentration measured in the multilevel mini-piezometer samples was only 2.53 mg l^{-1}. The highest coefficient of variation and range were observed with the DET gels (173.36 and 34.23 mg l^{-1}, respectively), however, the lowest coefficient of variation was found in the Minipoint samples (135.05) and the lowest range in the multilevel mini-piezometer samples (15.00 mg l^{-1}, Table 3). The coefficient of variation of the multilevel mini-piezometer data and the range of the Minipoint data were intermediate of these values (151.78 and 17.62 mg l^{-1}, respectively). There was, however, no statistically significant difference (p-value = 0.27, Table 2) in nitrate concentrations in the top 0.15 m between the methods used.

3.2.1.2. Pore-water ammonium

3.2.1.2.1. Vertical concentration profiles in the top 1 m of the streambed. The observed pore-water ammonium depth profiles varied between the three techniques (Fig. 3); with the largest values and range observed in samples from multilevel mini-piezometers, and the lowest concentrations observed with the Minipoints. Mean pore-water ammonium concentrations were determined at each sampling depth used for each method and the largest mean concentrations (3.83 to 5.73 mg l^{-1}) and range (1.90 mg l^{-1}) were observed in the multilevel mini-piezometer samples, and the smallest mean concentrations (0.50 to 1.56 mg l^{-1}) and range (1.06 mg l^{-1}) were observed in the Minipoint data (Fig. 4). Differences in pore-water ammonium concentrations between the three methods were statistically significant (p-value <0.01, Table 2), with significant differences between all sampling methods (all p-values <0.01, Table 2). The most pronounced trend in mean pore-water ammonium concentration with depth was observed in the Minipoint data, where concentrations increased linearly with depth from 0.50 to 1.56 mg l^{-1} (Fig. 4), and the multilevel mini-piezometer data indicated a maximum in pore-water ammonium concentration of 5.73 mg l^{-1} at 0.2 m.

3.2.1.2.2. Vertical concentration profiles in the top 0.15 m of the streambed. Descriptive statistics were calculated individually for each method from all data collected in the top 0.15 m of the streambed as this represents the overlap of the window of detection for the sampling methods. The highest mean pore-water ammonium concentration was observed in the multilevel mini-piezometer data (3.83 mg l^{-1}), whereas the lowest was observed in the Minipoint sampler data (1.05 mg l^{-1}). The mean pore-water ammonium concentration observed with the DET gels was intermediate of these values (2.32 mg l^{-1}). The coefficient of variation was highest in the Minipoint samples (188.57) and lowest in the multilevel mini-piezometer samples (74.67), whereas, the range was highest in the multilevel mini-piezometer data (11.64 mg l^{-1}) and lowest in the Minipoint data, with a similar range observed with the Minipoint samplers and DET gels (10.02 and 10.18 mg l^{-1}, respectively, Table 3). For the top 0.15 m, the differences in pore-water ammonium concentrations between the three methods were statistically significant (p-value <0.01, Table 2), and were significant between all sampling methods (all p-values <0.01, Table 2).

3.2.1.3. Surface water concentrations. Mean surface water nitrate concentrations were high (14.27 mg l^{-1}), whereas surface water ammonium concentrations were low (0.10 mg l^{-1}).

3.2.2. Laboratory experiments

A comparison of the mean pore-water ammonium concentration at each depth showed that the concentration in the DET gel samples was higher than in the multilevel mini-piezometer samples at all depths (Fig. 5). It should be noted, however, that pore-water ammonium concentrations were slightly higher in the multilevel mini-piezometer data than in the DET gel data in two samples (0.14 and 0.08 mg l^{-1} higher, high concentration solution, 25 mm depth). The differences in pore-water ammonium concentrations obtained by the two methods were statistically significant at all depths (p-value = 0.02, 0.02 and <0.01 for 2.5, 7.5 and 12.5 cm depths, respectively, Table 4). Pore-water nitrate concentrations were not measured during these laboratory experiments as no nitrate was detectable in the DET gel samples after processing. The ammonium concentrations in the DET gel samples, which were equilibrated in ultrapure water (as quality control), were below the limit of detection, and so were effectively zero.

3.3. Discussion

3.3.1. Field study

Despite the variations in pore-water concentrations observed using the different sampling techniques discussed in detail below, these differences were not statistically significant with respect to nitrate (p-value >0.54), suggesting that the choice of sampling techniques did not have a significant effect on the outcome of analysed pore-water concentrations. This would be expected given that the samplers do not all sample the same depths of the streambed and that they were not co-located and hence, the variability between different locations was

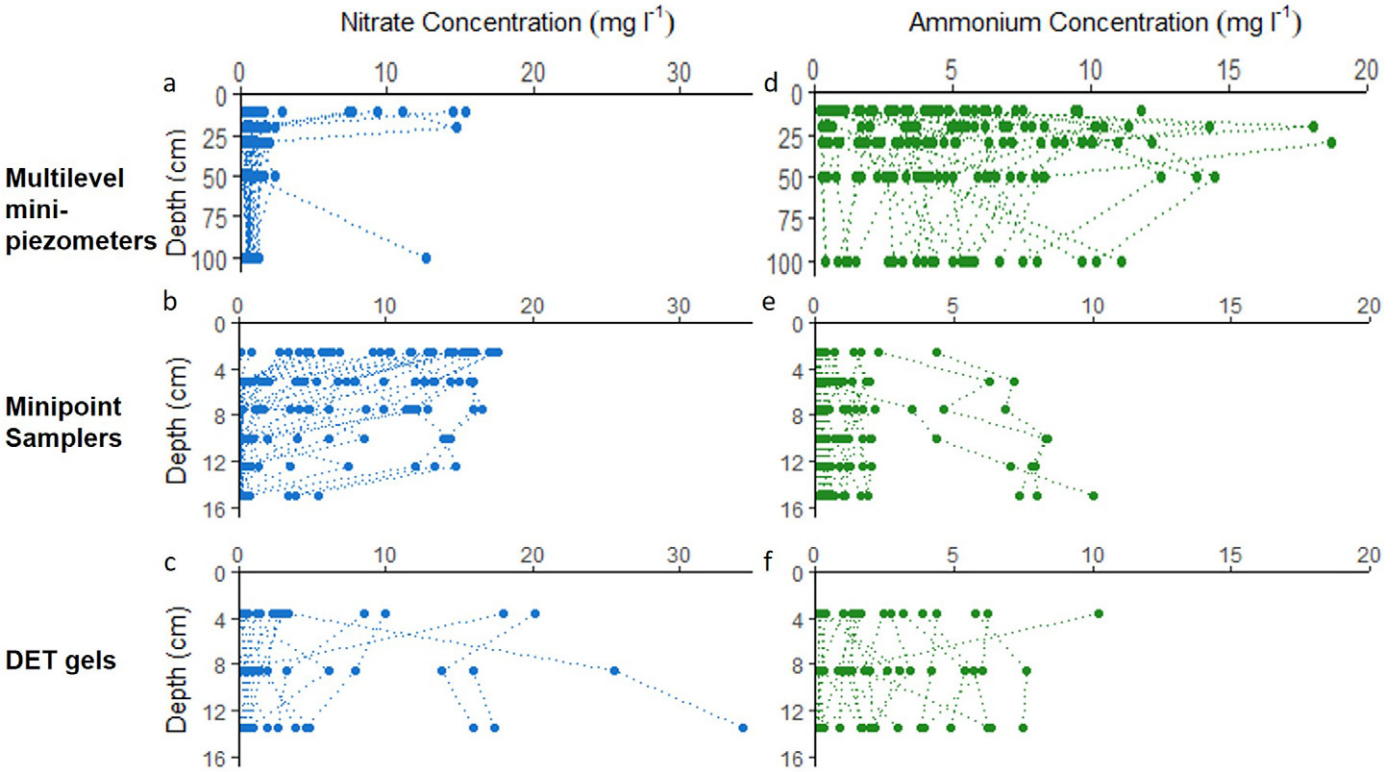

Fig. 3. Vertical profiles of pore-water nitrate concentration (mg l^{-1}) observed in the streambed of the Hammer Stream, Sussex, UK using a. multilevel mini-piezometers, b. Minipoint samplers and c. diffusive equilibrium in thin-film (DET) gel probes and vertical profiles of pore-water ammonium concentration (mg l^{-1}) in the streambed of the Hammer Stream, Sussex, UK using d. multilevel mini-piezometers, e. Minipoint samplers and f. DET gels.

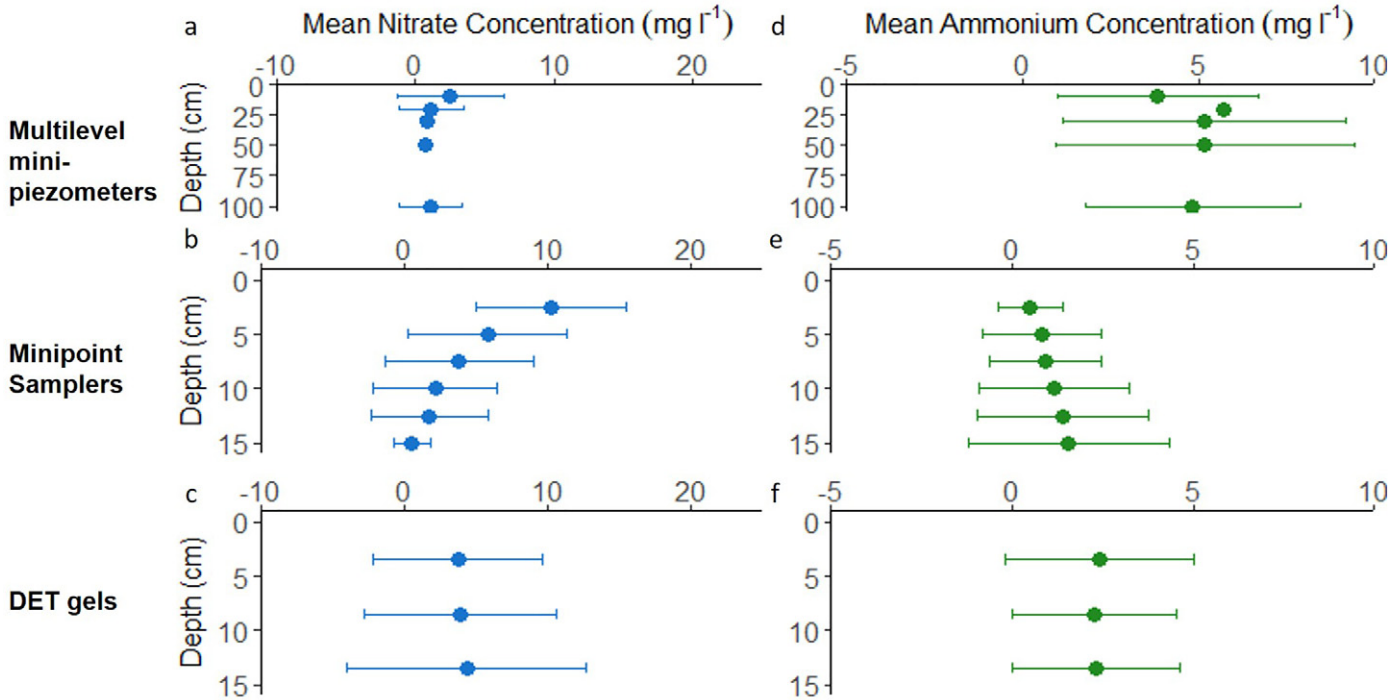

Fig. 4. Mean pore-water nitrate concentrations (mg l^{-1}) ±1 standard deviation for each sampling depth analysed in the streambed sediments of the Hammer Stream, Sussex, UK by using a. multilevel mini-piezometers, b. Minipoint samplers and c. diffusive equilibrium in thin-film (DET) gels and mean pore-water ammonium concentrations (mg l^{-1}) ±1 standard deviation for each sampling depth in the streambed sediments of the Hammer Stream, Sussex, UK using d. multilevel mini-piezometers, e. Minipoint samplers and f. DET gels.

Table 2
Statistical test results from all data from the Hammer stream, UK, where the Kruskal-Wallis rank sum test indicated a significant difference between results obtained by the different pore-water sampling methods, a Dunn test was used to determine which groups of pore-water samples were significantly different. Statistically significant comparisons are indicated by bold *p*-values.

Groups	*p*-Value	d.f.	Test
Nitrate	0.54	2	Kruskal-Wallis rank sum
Ammonium	<**0.01**	2	Kruskal-Wallis rank sum
DET-minipoint	<**0.01**	–	Dunn test
DET-piezometer	<**0.01**	–	Dunn test
Minipoint-piezometer	<**0.01**	–	Dunn test
Nitrate (15 cm)	0.27	2	Kruskal-Wallis rank sum
Ammonium (15 cm)	<**0.01**	2	Kruskal-Wallis rank sum
DET-minipoint (15 cm)	<**0.01**	–	Dunn test
DET-piezometer (15 cm)	<**0.01**	–	Dunn test
Minipoint-piezometer (15 cm)	<**0.01**	–	Dunn test

greater than the variability between techniques. Even though the differences were not statistically significant, there were differences observed and these affected biogeochemical classification of the streambed (see detailed discussion below), therefore, the methods used should be carefully chosen to capture the data required to address experimental hypotheses.

On the other hand, there was a statistically significant difference in pore-water ammonium concentrations (p-value <0.01) obtained by the different pore-water sampling techniques, indicating that the selected sampling technique can have wide implications for experimental results. It is somewhat surprising that there was no statistically significant difference in the pore-water nitrate concentrations, given that pore-water nitrate concentrations have been shown to be sensitive to active versus passive sampling techniques (Briggs et al., 2015). Although significant differences between these methodologies were observed, care should be taken when comparing results gained from differing sampling techniques.

The differences in concentrations measured with the three pore-water sampling techniques may be explained by some key differences in sampler principles and setup. The Minipoint samples revealed mean pore-water concentrations at the first sampling depth that were higher in nitrate and lower in ammonium concentrations than samples obtained from the multilevel mini-piezometers. However, as both techniques use active sampling methods, similar concentrations would be expected. The difference may be explained by the common multilevel mini-piezometer setup used, where pore-water is sampled at a coarser resolution over a larger depth range (Krause et al., 2013; Rivett et al., 2008). Here, the shallowest depth sampled with the multilevel mini-piezometers was 100 mm, therefore, any downwelling surface water, which is high in nitrate and low in ammonium at this site, would already have been affected by streambed processes occurring at shallow sampling depths (Battin et al., 2003b; Knapp et al., 2017; O'Connor and Harvey, 2008), whereas the Minipoint samples at 25 mm would capture this surface water signature more efficiently. Additionally, the volume sampled with the Minipoints was five times larger than that sampled with the multilevel mini-piezometers, which despite low pumping rates, may have increased hyporheic flow. This is furthermore evidenced by other research at this study site, which found that nitrate entering the streambed in surface water was immediately reduced (Shelley et al., 2017). The depth of sampling, with most of the multilevel mini-piezometer samples extracted from >0.3 m depth, may also explain why this technique resulted in the lowest pore-water nitrate concentrations and the highest pore-water ammonium concentrations, as a different section of the streambed is being sampled. The results here correspond with previous observations of significant rates of denitrification between depths of 50 mm and 0.7 m in streambed sediments (Stelzer et al., 2011); however, previous research at this site found low rates of nitrate reduction at depths >0.60 m (Shelley et al., 2017). It is important to note that multilevel mini-piezometers may be designed to sample at a finer resolution in the top 0.2 m of the streambed, with an achievable sampling resolution of 50 mm (Rivett et al., 2008).

Table 3
Descriptive statistics for all pore-water data from the top 0.15 m of the streambed obtained from application of DET gels, Minipoint samplers and multilevel mini-piezometers sampling at the Hammer Stream, Sussex, UK.

Method	Nitrate (mg l^{-1})			Ammonium (mg l^{-1})		
	Mean	CV	Range	Mean	CV	Range
Multilevel mini-piezometer	2.53	151.78	15.00	3.83	74.67	11.64
Minipoint sampler	4.08	135.05	17.62	1.05	188.57	10.02
DET gel	4.02	173.36	34.23	2.32	101.52	10.18

Analysis of the DET gel samples yielded different concentrations than samples obtained from Minipoints, despite these two techniques sampling similar depths within the streambed. Both samplers, however, are mechanically different; DET gels are passive samplers (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012) whereas the Minipoints are active samplers, hence Minipoints are likely to sample pore-water from more mobile macropores and the DET gels from micropores or matrix pores (Harvey, 1993; Harvey et al., 1995). The Minipoints may, therefore, predominantly sample mobile water (often downwelling surface water in the near-surface sediment), which primarily flows through the macropores, whereas, the DET gels should predominantly sample less mobile micropores less likely to reflect surface water concentrations. Macropores and micropores have differing characteristics with shorter residence times, more oxygenated conditions, lower rates of denitrification and higher rates of nitrification typically observed in macropores than micropores (Briggs et al., 2015), which may explain the higher pore-water nitrate and lower pore-water ammonium concentrations found in the Minipoint data.

Similar differences in ammonium concentrations in active versus passive samplers have been observed previously where larger ammonium concentrations were observed in DET gel samples than in multilevel mini-piezometer samples (Mortimer et al., 1998; Ullah et al., 2012); however, no differences have also been observed (Krom et al., 1994; Mortimer et al., 2002). This may also have affected the vertical profiles obtained from the Minipoints and the DET gels, with a non-linear decrease in pore-water nitrate and a linear increase in pore-water ammonium observed with depth in the Minipoint data, which was not seen with the DET gels. Despite the hypothesis presented here, more rigorous testing of the pore space sampled by active versus passive samplers is required to determine whether this accounts for the differences in ammonium concentrations observed between DET gels and active samplers.

Furthermore, as porewater was extracted using Minipoints the samples for laboratory analysis were extracted in-situ, however, as the DET gel only samples solutes diffused into the polyacrylamide gel, a solution has to be created for analysis in the laboratory using back-equilibration. This process could produce differences in pore-water concentrations between the two sampling techniques, especially given that here gel slices were back-equilibrated on ice for 20 h. The time required for back-equilibration was not tested here and so the time used (20h) may have been unnecessarily long, and is sufficient for potential changes in resulting pore-water concentrations to occur. Additionally, the difference in sampling resolution (25 mm in the Minipoints and 50 mm in the DET gels), may have had some effect on the vertical profile; however, it is difficult to interpret the effect due to the multidirectional nature of hyporheic flow (Bencala, 1993; Mulholland and DeAngelis, 2000).

These differences in sampler principles and setup may also have affected the vertical trends of nitrogen species observed, with the clearest trend observed in the Minipoint data. Minipoint samplers were able to sample the mobile pore-waters in the most biogeochemically variable upper zone of the streambed (Battin et al., 2003b; Knapp et al., 2017; O'Connor and Harvey, 2008; Shelley et al., 2017), allowing for influences

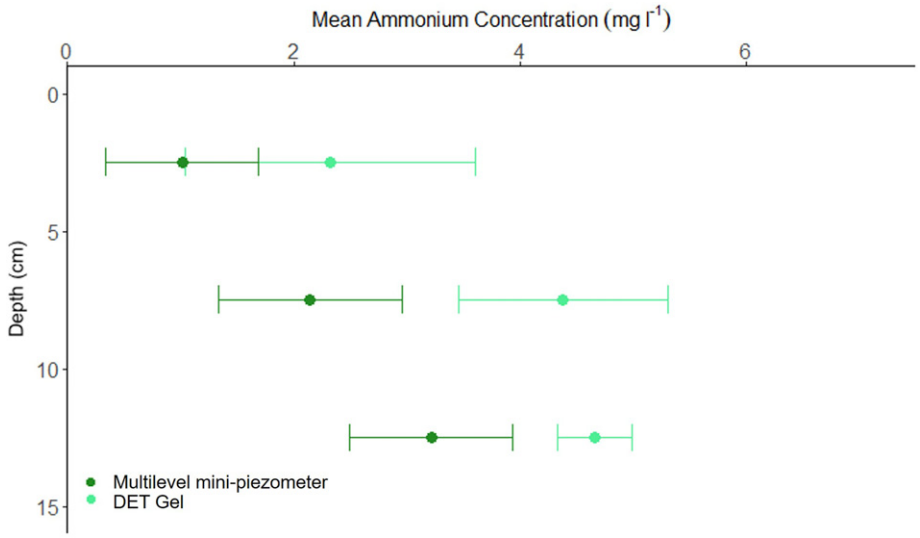

Fig. 5. Mean ammonium pore-water concentrations (mg l^{-1}) ±1 standard deviation found by multilevel mini-piezometer and DET sampling at each sampling depth in the laboratory column experiments.

of downwelling surface water and biogeochemical processes to be observed in the profile. The lack of trend in the DET gel data was unexpected, especially given that DET gels have previously been used to capture biogeochemically active zones within sediment (Comer-Warner et al., 2017; Ullah et al., 2012, 2014).

The samples collected using the investigated methods were not ideally co-located nor sampled simultaneously. Samples were collected from multilevel mini-piezometers at a different time (9th July 2015) than those from the DET gels (17th June 2015) and Minipoint samplers (16-18th June 2015), and the Minipoint samplers were not co-located with the DET gels and multilevel mini-piezometers (see Fig. 2c). Despite the sampling variations we believe the discussion remains valid due to co-located samplers requiring sufficient distance between them to prevent interference, therefore, even co-located samplers may not sample the same parcel of water. This is particularly important where there is large variability in nutrients at small-scales, which has been observed in the Hammer Stream (Shelley et al., 2017). The techniques were utilised individually to gather insight into the reach-scale streambed biogeochemistry inferred from nutrient profiles obtained from each method, therefore, all data from each sampling technique were compared rather than individual nutrient profiles. We believe the presented results are crucial observations of wider relevance, because outcomes from different sampling techniques are often used interchangeably without considering effects inherent to the technique. The quantitative comparison presented here, therefore, provides valuable information on the validity of assumptions that different sampling techniques provide comparable results.

The differences in results from the streambed samplers utilised in this case study may have resulted from variations in the window of observation, vertical resolution and sampler principles (active versus passive) between the methods. These differences may lead to conflicting characterisation of the biogeochemical conditions influencing streambed pore-water concentrations within the study reach; therefore, potentially different conclusions could be drawn based on the analysis of results from studies that apply only one method.

For the field case study presented here the streambed characterisation did vary between the methods used. The multilevel mini-piezometer samples indicated a stream reach characterised by reduced conditions and anoxia, leading to a decrease in pore-water nitrate and increase in pore-water ammonium (Dahm et al., 1998; Duff and Triska, 2000; Lansdown et al., 2016; Lansdown et al., 2014; Naranjo et al., 2015). This was reflected in the vertical profiles of mean pore-water concentration values obtained with the multilevel mini-piezometers, which indicated surface water high in nitrate and low in ammonium penetrating the subsurface. There was then a decrease in pore-water nitrate and increase in pore-water ammonium with depth (Fig. 4a and d). The DET gel data indicated a stream reach characterised by areas of oxygenated sediment, leading to a few points of high pore-water nitrate concentration (Dahm et al., 1998; Duff and Triska, 2000; Holmes et al., 1994; Jones Jr. et al., 1995; Naranjo et al., 2015; Seitzinger, 1994), within a streambed similar to that described in Section 3.1.1 for the multilevel mini-piezometer data. This perhaps contributed to the lack of trend in mean pore-water nitrate and ammonium concentrations with depth in the DET gel samples, with little vertical variation in mean pore-water concentrations making it difficult to infer biogeochemical process information (Fig. 4c and f).

In contrast, the Minipoint data indicated a stream reach characterised by oxidising conditions, leading to high pore-water nitrate and low pore-water ammonium concentrations (Dahm et al., 1998; Duff and Triska, 2000). The mean pore-water concentration profiles obtained from the Minipoints indicated a decrease in pore-water nitrate coupled with an increase in pore-water ammonium with depth (Fig. 4b and e). This is likely due to surface water, which is high nitrate and low ammonium concentration here, entering the streambed, before a decrease in pore-water nitrate and increase in pore-water ammonium at greater depths resulting from the majority of biogeochemical processing occurring in the upper few centimetres of sandy or fine-grained sediments (Battin et al., 2003b; Knapp et al., 2017; O'Connor and Harvey, 2008; Shelley et al., 2017), which are characteristic of the study site (Shelley et al., 2017).

The streambed characterisation was likely affected by differences in sampler set-up and principles. The window of detection and vertical resolution varied between sampling methods with multilevel mini-piezometers sampling at greater depths and over a wider range (0.1 to 1 m) than the Minipoints (0.025 to 0.15 m) and the DET gels (0.035 to 0.135 m), while the Minipoint samplers had the highest vertical

Table 4
Statistical test results from all pore-water data from the laboratory column experiments, *p*-values <0.05 (shown in bold) indicate a significant difference between pore-water samples extracted by DET gels and multilevel mini-piezometers at the respective depths.

Groups	*p*-Value	d.f.	Test
DET gel v piezometer 2.5 cm	**0.02**	–	Wilcoxon signed rank
DET gel v piezometer 7.5 cm	**0.02**	–	Wilcoxon signed rank
DET gel v piezometer 12.5 cm	<**0.01**	8	Paired *t*-test

resolution (25 mm) compared to the DET gels (50 mm) and the multilevel mini-piezometers (0.1 to 0.5 m, depending on depth). This resulted in the majority of the multilevel mini-piezometer data originating outside the top, biogeochemically reactive layer of the streambed, whereas all of the data from the Minipoints and DET gels were collected from within the top 0.15 m. Additionally, the higher vertical resolution of the Minipoint data, and to a lesser extent the DET gel data, allows small-scale pore-water concentration dynamics to be observed. These combined may explain why pore-water nitrate was lower and pore-water ammonium was higher in the multilevel mini-piezometer samples, as these concentration dynamics are often also observed with increasing depth below the sediment surface where typically anoxia increases and is therefore, accompanied by an increase in denitrification and decrease in nitrification (Dahm et al., 1998; Duff and Triska, 2000). The difference in sampling resolution utilised in the top 0.15 m of the streambed enabled clearer trends in nutrient depth profiles to be determined in the Minipoint data than in the DET gel data.

As discussed in Section 3.3.1, the difference in sampler principles between Minipoints and DET gels, i.e. active versus passive sampling, likely also influenced the streambed characterisation, resulting in DET gels preferentially sampling different pore-waters to the Minipoints. This explains the higher pore-water ammonium concentrations and the lower pore-water nitrate concentrations in the top sampling depths observed in the DET gels than the Minipoints. The pattern may also be explained by increased surface water downwelling induced by sampling with the Minipoints at too high sampling volumes or rates relative to the natural flow, although here care was taken to avoid this scenario. Additionally, the variability in observed concentrations may be enhanced by the upwelling that was observed locally with the Minipoint samplers at three locations, whereas surface water was downwelling at all other locations.

The differences in behaviour between pore-water nitrate and ammonium profiles observed are expected due to the fundamental differences in biogeochemical processes that each nutrient experiences. Ammonium and nitrate are involved in many redox reactions but are predominantly affected by differing redox conditions in streambeds and will, therefore, be present at varying concentrations depending on oxygen availability (Bollmann and Conrad, 1998; Davidson, 1991; Heppell et al., 2013; Lansdown et al., 2012, 2015; Quick et al., 2016; Well et al., 2005). Furthermore, the sorption of ammonium to clay sediment produces additional controls on the availability and fate of ammonium (Duff and Triska, 2000), which does not directly affect nitrate.

3.3.2. Laboratory experiment

The laboratory experiment allowed further investigation of the effect of active versus passive sampling on resulting ammonium concentrations that was observed in the in-situ data. The ammonium concentrations observed in the data from the DET gels were greater than those observed in the samples obtained from the co-located multilevel mini-piezometers in all three ammonium sediment concentrations used (Fig. 5), which has been observed previously (Ullah et al., 2012). We believe that the discrepancy between techniques, between 31 and 56% over the different depths in this experiment, is further evidence of the difference in sampling principles between active and passive samplers. DET gels equilibrated in ultrapure water resulted in ammonium concentrations below the limit of detection (0.02 mg N l^{-1}) and confirmed that the high pore-water ammonium concentrations observed in the DET gels during the in-situ or laboratory experiments were not introduced from the DET gels themselves.

As mentioned in Section 2.2.1, the DET gel is a passive, diffusive equilibrium sampler (Byrne et al., 2015; Krause et al., 2011a; Ullah et al., 2012) sampling micropores, whereas, the multilevel mini-piezometers are active samplers relying on a vacuum or pumping action to sample the 'free' pore-water that occupies macropores. The DET gels preferential sampling of micropores/matrix pores (Harvey, 1993; Harvey et al., 1995) can explain the large differences in pore-water ammonium concentrations found between the two methodologies due to active and passive samplers sampling different pore-waters and therefore, different chemical signatures, as outlined in detail in Section 3.3.1.

The difference in pore-water ammonium concentrations observed between the data from the DET gels and the multilevel mini-piezometers was statistically significant (p-values <0.05) indicating that the principles of the sampling methodology (active versus passive) used can greatly influence the resulting concentration of ammonium. When designing an experiment, the researcher should, therefore, carefully consider whether they need to target macropores or micropores to address their research questions, or if they need to utilise a combination of both active and passive sampling methods. Furthermore, the methods discussed in this paper are all ex-situ in nature, i.e. samples are collected from the streambed and analysed in the laboratory. In-situ pore-water chemistry measurement methods are also available, and continue to be developed, these methods have the advantage of capturing the intended concentration dynamics without issues of contamination or concentration changes associated with transport, storage and laboratory analysis. These methods should, therefore, also be considered during experimental design.

4. Conclusions

As interest in hyporheic biogeochemistry continues to increase, along with the volume of interdisciplinary research conducted in the HZ, the development of standard sampling protocols and further sampling methods is required. The three samplers (multilevel mini-piezometers, Minipoint samplers and DET gels) discussed in this study mainly differ with respect to the absolute sampling depth they can reach, the achievable vertical spatial resolution and the pore sizes (and therefore mobile versus immobile water) samples are predominantly extracted from. Disturbances in subsurface flow may also vary between sampling techniques depending on sample volumes and sampling rates used for active sampling and the relatively large horizontal sampler footprint of the DET gel, which may affect nutrient profiles near the sediment-water interface where strong gradients are observed.

Although samplers such as Minipoints and DET gels provide high-resolution nutrient profiles in the top few centimetres of the streambed, where the majority of biogeochemical cycling occurs, multilevel and single-depth piezometers remain a valuable tool for the investigation of deeper influences of groundwater and larger scale processes. The extent of hydrological information and the macropore versus matrix zones sampled also vary with technique, therefore, care needs to be taken when selecting a methodology. Furthermore, the sampling method used may significantly affect the resulting ammonium concentrations and may result in differing conclusions on reach-scale streambed characteristics (Table 5). The research question, and desired spatial and temporal resolution will, therefore, determine which sampling technique is most appropriate to use, with each one characterised by specific advantages and limitations (Table 1). Larger scale processes including groundwater zones of upwelling and downwelling, hydrological information and contaminant plume identification and investigation at greater depths are best investigated using multilevel or single depth mini-piezometers, which allow chemical and hydrological information to be determined at the same point within macropores at greater depths. The ability to also sample at shallower depths allows processes within the shallow streambed to be investigated although at a coarser resolution than miniature drivepoint samplers. In-depth characterisation of hyporheic zone hydrology and biogeochemical processes in the top 0.4 m of the streambed are best investigated using miniature drivepoint samplers, which allow high-resolution investigation of chemical and hydrological information at the same

Table 5
Summary results of the in-situ field comparison of nitrate and ammonium pore-water concentrations obtained from multilevel mini-piezometers, Minipoint samplers and DET gels, as well as suggested applications for the respective pore-water sampling techniques.

Sampling methodology	Nitrate	Vertical nitrate profile	Ammonium	Vertical ammonium profile	Streambed redox conditions	Applications
Multilevel mini-piezometers	Low	Decrease with depth, although not very pronounced	High	Maxima at 0.2 m	Reduced	- Coarser investigation of exchange processes and biogeochemical activity within the wider streambed - Determination of hydrological characteristics and reaction rates a wide range of depths (up to a few metres) - Investigation of coarser resolution (50–100 mm) nutrient and contaminant dynamics throughout the streambed - Detection and investigation of groundwater and associated contaminants
USGS Minipoint Samplers	Low-high	Non-linear decrease with depth	Low with some high	Linear increase with depth	Oxidised	- Fine scale investigation of exchange processes and biogeochemical activity within the hyporheic zone - Determination of hydrological characteristics and reaction rates within the top 0.4 m of the streambed - Investigation of high resolution (10–30 mm) nutrient and contaminant dynamics in the top 0.4 m of the streambed
DET gels	Low with some high	No obvious shape to the profile	Intermediate	No obvious shape to the profile	Reduced with oxic zones	- Fine scale investigation of biogeochemical processes within the hyporheic zone - Investigation of very high resolution (1 mm–10 mm) nutrient and contaminant dynamics in the top 0.15 m of the streambed

depth within macropores. Fine-scale investigations of concentration dynamics within the top 0.15 m of the streambed are best investigated using DET gels, which allow very high vertical resolution measurements of the sediment matrix of micropores, but no hydrological information to be obtained, although the passive nature of this technique means it may be difficult to capture some events.

The differences between pore-water sampling methodologies presented here provide guidance for future studies into pore-water nitrogen cycling, improving sampler selection based on specific research questions. This has global relevance for researchers focussing on important questions of chemical cycling within saturated sediments including the hyporheic zone, moving towards a more uniform sampling protocol and better understanding of how the selected methodology may bias results.

Future work is needed to develop sampling methodologies with focus on in-situ methodologies that measure nutrient concentrations without the need for sample extraction, therefore, reducing the likelihood of results being altered by the sampling technique. Ex-situ methodologies, such as those examined here, continue to be of importance and further development of these methods including high vertical resolution samplers robust enough to sample gravels and cobbles is encouraged.

Declaration of competing interest

The authors declare no conflicts of interest, either financial or personal, that may be perceived to influence this work.

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